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THE SUPRASPINAL NEURAL CORRELATE OF BLADDER COLD SENSATION—AN FMRI STUDY

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ABSTRACT

In recent years, functional imaging studies have revealed a supraspinal network, which is involved in perception and processing of bladder distention. Very little information exists on the cortical representation of C-fiber transmitted temperature sensation of the human bladder, although C-fibers seem to be involved in the pathomechanisms of bladder dysfunctions. Our aim was, therefore, to evaluate the outcome of bladder cold stimulation on supraspinal activity using functional magnetic resonance imaging (fMRI). A block design fMRI study was performed in 14 healthy females at the MR-center of the University of Zurich. After catheterization, all subjects were investigated in a 3.0- Tesla Scanner. The scanning consisted of 10 repetitive cycles. Each cycle consisted of five conditions: REST, INFUSION, SENSATION, DRAIN 1, and DRAIN 2. Cold saline was passively infused at 4–8°C during scanning. Not more than 100 ml were infused per cycle. Blood-oxygen-level-dependent (BOLD) signal analysis of the different conditions was compared to REST. All activations were evaluated on a random effects level at $P = 0.001$. Activation of brain regions for bladder cold stimulation (DRAIN 1 period) was found bilaterally in the inferior parietal lobe (Brodmann area (BA) 40), the right insula (BA 13), the right cerebellar posterior lobe, the right middle temporal gyrus (BA 20), and the right postcentral gyrus (BA 3). In conclusion, bladder cooling caused a different supraspinal activation pattern compared to what is known to occur during bladder distention. This supports our hypothesis that cold sensation is processed differently from bladder distension at the supraspinal level.

Keywords: bladder cooling; bladder sensations; C-fibers; fMRI; supraspinal control

INTRODUCTION

There are two main afferent fiber types in the lower urinary tract (LUT), A-delta (Aδ) and C-fibers. The myelinated Aδ-fibers are known to transmit signals of bladder wall distension and therefore, to indicate the degree of bladder filling [1, 2]. The unmyelinated C-Fibers are thought to transmit signals caused by inflammation, thermal stimuli (e.g., cold), and noxious or chemical stimuli (e.g., vanilloids, resinferatoxin, and changes in urine pH;[1-3]].

The influence of Aδ-fiber transmitted information on the activation of supraspinal neuronal structures has been investigated in recent years during different procedures of bladder filling and emptying using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI; [4]]. These studies have revealed a complex supraspinal network, involved in the functioning and control of the lower urinary tract (LUT), which includes frontal and orbito-frontal areas, the anterior cingulate gyrus (ACG), the insula, the thalamus, the cerebellum, the pons, and the periaqueductal gray (PAG; [4]].

Still, little is known about the central neural correlates of bladder C-fiber stimulation. In healthy subjects with a normal LUT function, C-fibers are regarded as silent [1, 2, 5].

However, there are certain neurological (e.g., spinal cord injury and multiple sclerosis) and other, rather locally confined pathological LUT conditions (e.g., inflammation, overactive bladder, and painful bladder syndrome) that are associated with changes in the sensitization and sprouting of bladder C-fibers [1, 2, 6-8]. Thus, changes in C-fiber activity have been regarded as part of the pathophysiology of LUT dysfunctions, such as increased urination frequency and urgency, as well as suprapubic pain [7].

Therefore, investigation of C-fiber activation and its central nervous system representation are of relevance for understanding LUT function and dysfunctions.

As C-fibers are sensitive to cold stimulation [1, 3, 5], bladder cooling with rapid cold saline infusion into the bladder would be a safe and feasible way to stimulate C-fibers during functional brain imaging. Although cooling is not considered a physiological sensation normally arising from the bladder, it has previously been demonstrated to be an useful diagnostic method for investigating functional LUT disturbances [7, 9-11] and may also be a good paradigm for studying painful processes in the LUT associated to C-fiber stimulation [7].

Our aim in this study was to investigate the supraspinal neural correlate of bladder cold perception using fMRI in healthy subjects. On the basis of the findings of a previous PET study [12], we hypothesized that bladder cooling recruits a different cerebral network than bladder filling.

MATERIALSAND METHODS

After approval from the local ethics committee, 14 healthy female subjects gave their written informed consent for participating in the study. Inclusion criteria were as follows: healthy, right-handed females, aged 18–35 years. Exclusion criteria were: any actual health problems, urinary tract infection (UTI),

latex allergy, previous or current pregnancy, symptoms of overactive bladder (OAB) or other LUT symptoms, and ferromagnetic implants.

We included only female subjects for reasons of group homogeneity. Although men and women seem to have a similar overall prevalence for lower urinary tract symptoms like OAB, they show distinct differences in certain symptoms, which might be related to the different anatomy of the male and female LUT [13]. In addition, our experience has shown that catheterization is much less uncomfortable for female subjects than for males.

IMAGE ACQUISITION

The study was performed in the MR (magnetic resonance)-Center of the University of Zurich, using a 3.0-Tesla Scanner (Philips, Achieva) and an eight-element head coil.

Functional BOLD sensitive images were acquired using a single-shot gradient echo EPI pulse sequence (TE/TR = 35/3,000 ms, flip angle = 82°, FOV = 220 × 220 mm^2, matrix = 128 × 128, slices = 39, slice thickness = 3 mm). Sensitivity encoding (SENSE) with a reduction factor of two was used to minimize the influence of susceptibility artifacts and to maximize the possible number of slices acquired within one TR.

High-resolution anatomical images were acquired before the functional experiments, using a 3D T1-weighted gradient echo sequence (TE/TR = 2.3/20 ms, FOV = 220 × 220 mm^2, matrix = 256 × 256, slices = 180, slice thickness = 0.75 mm). All images were obtained in an oblique axial orientation covering the entire head.

EXPERIMENTAL DESIGN

Following transurethral catheterization using a nonanesthetic lubricant and a soft 14-Fr latex catheter, the individual bladder capacity of each subject was determined by manually filling the bladder until strong desire to void (SDV).

TEST RUN

A test run was performed prior to the actual fMRI measurements consisting of 10 repetitive cycles with 5 different conditions per cycle: tREST, tINFUSION, tSENSATION, tDRAIN 1, and tDRAIN 2 (t = test run condition; **Table 1**). During tREST, no specific stimulus was given and the bladder was empty. During tINFUSION, the bladder was passively filled with cold saline (4–8°C) and the time until subjects indicated the beginning of bladder cold sensation was recorded for each cycle. tSENSATION started per definition, when subjects indicated the very first sensation of bladder cooling. During tSENSATION, cold saline was still infused into the bladder. With the start of tDRAIN 1, the infusion stopped and the bladder was drained. During tDRAIN 2, the bladder was empty again. The repetitive, passive infusion and drainage of the cold saline was realized through tubes connected to the

transurethral catheter via a simple Y-connector (see **Figure 1**). In- and outflow was regulated via two valves, one on the inflow tube (Valve 1) and one on the outflow tube (Valve 2; see **Figure 1**), i.e., Valve 1 open and Valve 2 closed ¼ bladder fills with cold saline. Valve 1 closed and Valve 2 open = bladder drains and starts to warm-up (**Table 1**).

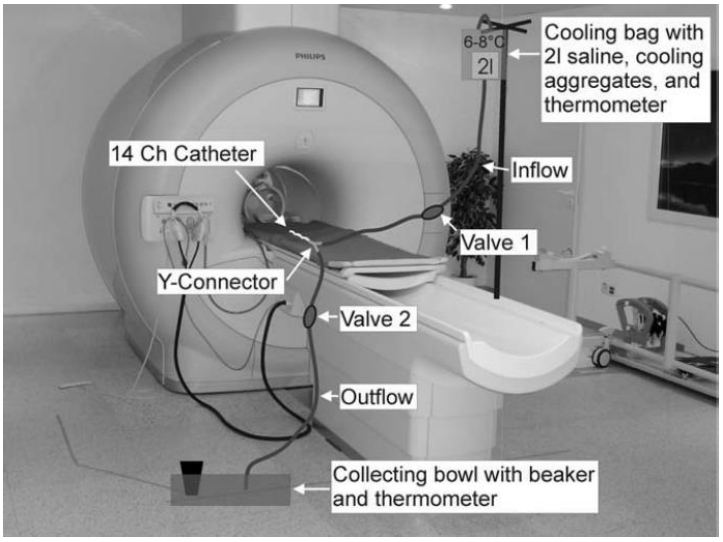

Figure 1 Experimental setup in the magnetic resonance (MR) facility.

Table 1 Status of the in- and outflow valves, the bladder filling, and the subjective sensations during the test runprior to the MR-scanning (t 5 test run condition).

	tREST	tINFUSION	tSENSATION	tDRAIN 1	tDRAIN 2
Duration	12 s	variable, mean of all subjects: 9.8 ± 1.9 s (range: 6–12 s)	12 s	30 s	30 s
Status of the valves at the in- and outflow tube	Valve 1 closed, Valve 2 open	Valve open, Valve 2 closed	Valve open, Valve 2 closed	Valve 1 closed, Valve 2 open	Valve 1 closed, Valve 2 open
Filling status of the bladder	Bladder is empty	Start of bladder filling with cold saline (4–8°C)	Bladder filling with cold saline (4–8°C)	Bladder is drained	Bladder is empty
Subjective sensation	No bladder cold sensation	No bladder cold sensation	First sensation of bladder cooling (= start of SENSATION), which slowly increases with further filling	Further increase in cold sensation up to 12 s, followed by a continuous decline in bladder cold sensation	Slight bladder cold sensation, which further declines until it is completely absent

The test run was performed to give the subjects an impression of how the cold stimulation feels like, and to determine their subjective sensations and the individual mean time until the cold sensation is perceived after starting INFUSION.

Because of the small catheter diameter, no more than 100 ml could be infused in total, which was controlled with a measuring jug, collecting the drained fluid from the bladder (see **Figure 1**).

Regarding the subjective sensations during the test run, we documented the following (**Table 1**): During tREST and tINFUSION, no bladder cooling was indicated. The tINFUSION condition had different durations, as different subjects indicated the start of bladder cold sensation (=start of tSENSATION) at different time points. We used the mean time of the 10 repetitions of tINFUSION in each subject to individually calculate the actual fMRI protocol.

With the beginning of tSENSATION, bladder cold sensation started. All subjects were able to feel cooling within the bladder, which they described as a deep and diffuse coldness that spreads and intensifies with further filling. The sensation of bladder cooling was described as being similar to the sensation felt in the stomach when drinking ice cold water or lemonade except that the location was different, being perceived more inferior in the lower pelvis, just above the pubic bone.

During tDRAIN 1, when the bladder started to drain and no further ice water was infused, the bladder cold sensation still increased during the first 10–12 s of this condition. This increase in cold sensation was followed by an initially slow, and subsequently faster, continuous decline of the sensation. During tDRAIN 2, the bladder was empty and the cold sensation declined further and finally disappeared completely. It took ~45–50 s after starting to drain the cold saline, until the subjects reported a complete disappearance of any cold sensation.

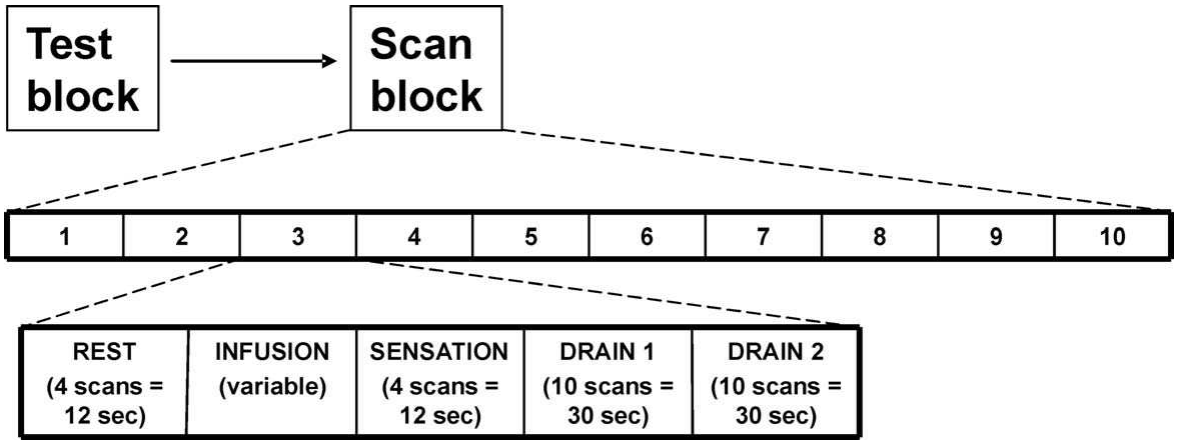

Figure 2 Scanning paradigm of the block design used in this study, indicating the number of repetitions (n = 10) and the sequence of the different scan conditions during each repetition. The repetitions were performed successively without pause.

FMRI MEASUREMENTS

For the fMRI measurements, subjects were placed supine into the scanner and positioned as comfortably as possible. Isolating foam pads on the hip and thigh of the subjects were used to ensure that the in- and outflow tubes did not touch the legs or any other body part of the subjects during the functional scans to reduce artifacts and/or somatosensory activations of the touched body region.

The fMRI was performed following the same paradigm as was used for the test run, with 10 repetitive cycles and 5 different conditions per cycle, namely REST, INFUSION, SENSATION, DRAIN 1, and DRAIN 2 (see **Figure 2**). Based on the subjective sensation and timing information from the test run,

the fMRI protocol was adapted to the individual mean time of bladder cooling sensation (**Table 1**, **Figure 2**). The repetitive bladder cooling was performed in the same way as described for the test run.

After completion of all scans, subjects had to indicate on a visual analog scale (VAS) the degree of comfortableness and arousal during the scans. A score of 10 on the VAS indicated ''very comfortable/pleasant'' or ''very calm/low arousal'' and a score of -10 indicated ''very uncomfortable/ painful'' or ''very excited/high arousal.''

FMRI DATA ANALYSIS

The preprocessing of the functional MRI data was performed using BrainVoyager 1.8 (Brain Innovation B.V., Maastricht, The Netherlands) and included motion correction, 4-mm spatial smoothing, high pass linear trend removal, and temporal filtering (three cycles in time course).

Following motion correction, 2 of the 14 acquired datasets had to be excluded from the final group analysis due to head motion >1.5 mm. Therefore, results (**Table 2** and **Table 3**, **Figure 3** and **Figure 4**) are shown for 12 subjects.

Next, motion-corrected data were normalized by interpolation of the 3D anatomical images to iso-voxel size ($1 \times 1 \times 1$ mm^3) and subsequent transformation into the Talairach space [14]. The Talairach coordinates give similar results to the MNI coordinates.

The functional data were then coregistered to the individual anatomical data, using a fully automatic, highly precise, alignment as implemented in the BrainVoyager software package.

The Talairach transformed contrast images were entered into a group-level random effect analysis [15], i.e., a two-level procedure of a full mixed-effects model, to generalize the activation to the population level. This analysis was based on the general linear model (GLM) and used a Gaussian hemodynamic response function (HRF) with commonly used time parameters: 5 s to response peak and 15 s to undershoot peak. The images of all experimental conditions (REST, INFUSION, SENSTION, DRAIN 1, and DRAIN 2) of the first-level were carried on to the second-level random-effects group analysis. Group contrasts were defined by subtraction logic (e.g., {-1 0 0 1 0} to calculate the difference between DRAIN 1 and REST). INFUSION, SENSATION, DRAIN 1, and 2 were separately contrasted to the REST condition.

All (de-)activations are presented as group results evaluated on random effects level with $t = 4.4$ ($P = 0.001$, uncorrected), overlaid on the group averaged anatomical image. In addition, we applied a cluster extent threshold correction, considering only clusters ≥50 contiguous voxel.

A single subject analysis was performed for smaller areas like PAG and pons with $P \leq 0.05$ (false discovery rate (FDR) corrected). For all experimental conditions, we evaluated the BOLD signal changes in the activated regions from the contrast DRAIN 1–REST. The BOLD signal changes were extracted by drawing a sphere (diameter of 10 mm) around the peak activation in a given area. To obtain a higher temporal resolution (i.e., smaller than a TR of 3 s) of the BOLD signal changes, the

data between two consecutive data points was interpolated as implemented in the BrainVoyager software package.

Table 2 Activated (positive *t* values) and deactivated (negative *t* values) brain regions during the different scanning conditions.

Condition	Hemisphere	Region	Brodmann area	Talairach coordinates			Cluster size	Peak activation $t = 4.4$
				x	*y*	*z*		
Infusion	Right	Precentral gyrus (frontal lobe)	4	57	–7	26	63	–6.3
		Angular gyrus (parietal lobe)	39	41	–59	39	110	–6.4
		Cerebellum, posterior lobe		36	–72	–41	73	–6
		Superior frontal gyrus (frontal lobe)	8	23	28	51	200	–7.1
		Superior frontal gyrus (frontal lobe)	10	7	59	24	378	–6.2
		Superior frontal gyrus (frontal lobe)	8	2	45	45	197	–5.8
	Left	Cerebellum, anterior lobe		–10	–54	–19	266	–7.2
		Medial frontal gyrus (frontal lobe)	10	–12	48	12	158	–6.2
Sensation	Right	inferior parietal lobe	40	54	–39	49	68	6.8
		middle temporal gyrus	39	41	–58	7	154	–5.7
		middle temporal gyrus	39	40	–71	14	77	–5.2
		middle temporal gyrus	19	35	–62	17	184	–6.9
		superior parietal lobe	7	28	–48	56	170	–5.9
		frontal lobe, sub-gyral	6	26	–1	56	214	–7.5
Drain 1	Right	Cerebellum, posterior lobe		24	–45	–37	495	8.7
		Cerebellum, posterior lobe		27	–63	–36	1021	8
		Cerebellum, posterior lobe		39	–50	–37	81	5.8
		Insula	13	36	–3	8	77	5.4
		Cerebellum, posterior lobe		50	–48	–20	63	5.1
		middle temporal gyrus	20	51	–33	–9	153	5.8
		postcentral gyrus	3	61	–17	31	86	5.9
		inferior parietal lobe	40	61	–33	29	555	7.4
		inferior parietal lobe	40	48	–38	43	2310	8.4
		Angular gyrus (parietal lobe)	39	35	–58	37	1973	7
	Left	inferior parietal lobe	40	–37	–55	39	337	6.6
		inferior parietal lobe	40	–45	–49	40	160	5.6
		inferior parietal lobe	40	–50	–48	47	51	5.7
Drain 2	Right	no activations or deactivations						
	Left	no activations or deactivations						

All activations and deactivations are shown as random effects with $t = 4.4$ ($P = 0.001$).

RESULTS

All 14 subjects (mean age: 24.8 years, range 21–34 years) included in the study tolerated the catheterization and the fMRI experiment well and indicated no pain during the study. The results present data of 12 subjects, as the datasets of two subjects had to be excluded from the analysis due to overly head motion (>1.5 mm).

Mean comfortableness and arousal scores on the VAS during cold water infusion were -0.8 ± 1.3 and 1.7 ± 3.4, respectively.

The average bladder capacity at SDV was 579.7 ± 179.2 ml. The average time until cooling of the bladder was perceived during the test run was 9.8 ± 1.9 s (range: 6–12 s). The average saline

temperature during INFUSION was (6.1 ± 0.8)°C (range: 4–8°C). The average saline temperature after drainage from the bladder at the beginning of DRAIN 1 condition was (18.3 ± 2.1)°C (range: 11.9– 23.7°C).

Table 3 Individual t-values and Tailarach coordinates for Pons, PAG, and thalamus activations in the 12 subjects.

	FDR correction 0.05; Cluster threshold 10 voxel		*Pons*			*PAG*			*Thalamus*		
Subject number	*t* value	*P* value	*x*	*y*	*z*	*x*	*y*	*z*	*x*	*y*	*z*
1	3.17	0.0015	6	–26	–23				4	–11	–1
			–9	–28	–18						
			–11	–19	–29						
2	2.28	0.023	–6	–22	–23	–14	–24	–2	14	–19	4
			–8	–25	–33	11	–25	–2	–14	–19	6
			9	–14	–20						
3	2.32	0.02	–8	–23	–29	–6	–19	–9	–12	–22	14
			11	–22	–30				–12	–19	9
									–17	–19	1
4	2.85	0.0043	–8	–29	–27	–5	–24	–9	–18	–10	7
			6	–29	–18	–1	–18	–22	17	–16	15
5	2.49	0.013	–3	–17	–17	6	–25	–12	–15	–20	15
						–1	–19	–9	–6	–5	0
									–12	–2	10
6	2.52	0.012	8	–28	–30	–6	–15	–6	5	–1	2
			–9	–19	–27	8	–15	–8			
			–1	–19	–21						
7	2,47	0.014	–9	–23	–24	8	–11	–6	6	–17	7
			–2	–28	–33				–5	–15	11
			10	–28	–36						
8	2.79	0.0052	3	–26	–26				–13	–14	5
			–6	–26	–20				22	–22	0
9	2.85	0.0044	–4	–24	–27	–4	–19	–5			
10	2.58	0.01	–2	–27	–19	–6	–16	–9			
			–2	–16	–23	9	–16	–8			
11	2.57	0.01				–4	–19	–15	16	–10	16
									–15	–24	16
12	2.9	0.0038									

FDR = false discovery rate

The INFUSION condition showed only deactivations, mainly in the right frontal lobe (Brodmann area (BA) 8 and 10) and cerebellum (**Table 2**). In the subsequent SENSATION condition, activations were found in the right inferior parietal lobule (IPL, BA 40). Deactivations were observed mainly in the right middle temporal gyrus (BA 19, 39), the right frontal lobe (BA 6), and the right superior parietal lobule (BA 7; **Table 2**).

The DRAIN 1 condition elicited activations in the IPL (BA 40) bilaterally, the right insula (BA 13), the right cerebellar posterior lobe, the right middle temporal gyrus (BA 20), and the right post-central gyrus

(BA 3; **Table 2**, **Figure 3a–c**). In the DRAIN 2 condition, neither deactivations nor activations were detected (**Table 2**).

The behavior of the BOLD signal change (%) during each condition confirmed that our scan paradigm was chronologically accurate (see **Figure 4**). **Figure 4** demonstrates that all activated areas show a remarkably similar BOLD signal change during the different experimental conditions, i.e., a low response during REST and INFUSION, an increasing response during SENSATION, a maximal response during DRAIN 1, and a low response again during DRAIN 2.

Activation of the Pons and PAG were not observed in the group analysis during any condition, even with very low *t* values. However, in the single subject analysis 10 of 12 subjects and 9 of 12 subjects showed significant activation in the pontine and PAG region, respectively (**Table 3**).

Significant thalamic activation was found in 9 of 12 subjects in the single subject analysis but not in the group analysis (**Table 3**).

Although frontal activation was not observed in the group analysis, each subject showed some scattered frontal activation (mainly in the superior and middle frontal lobes, BA 8, 9, 10, and 46) in the single subject analysis.

ACG activation, which was also not detected in the group analysis, was observed in 6 of 12 subjects. Those ACG activations were rather small clusters, surrounded by several larger clusters with significant deactivation.

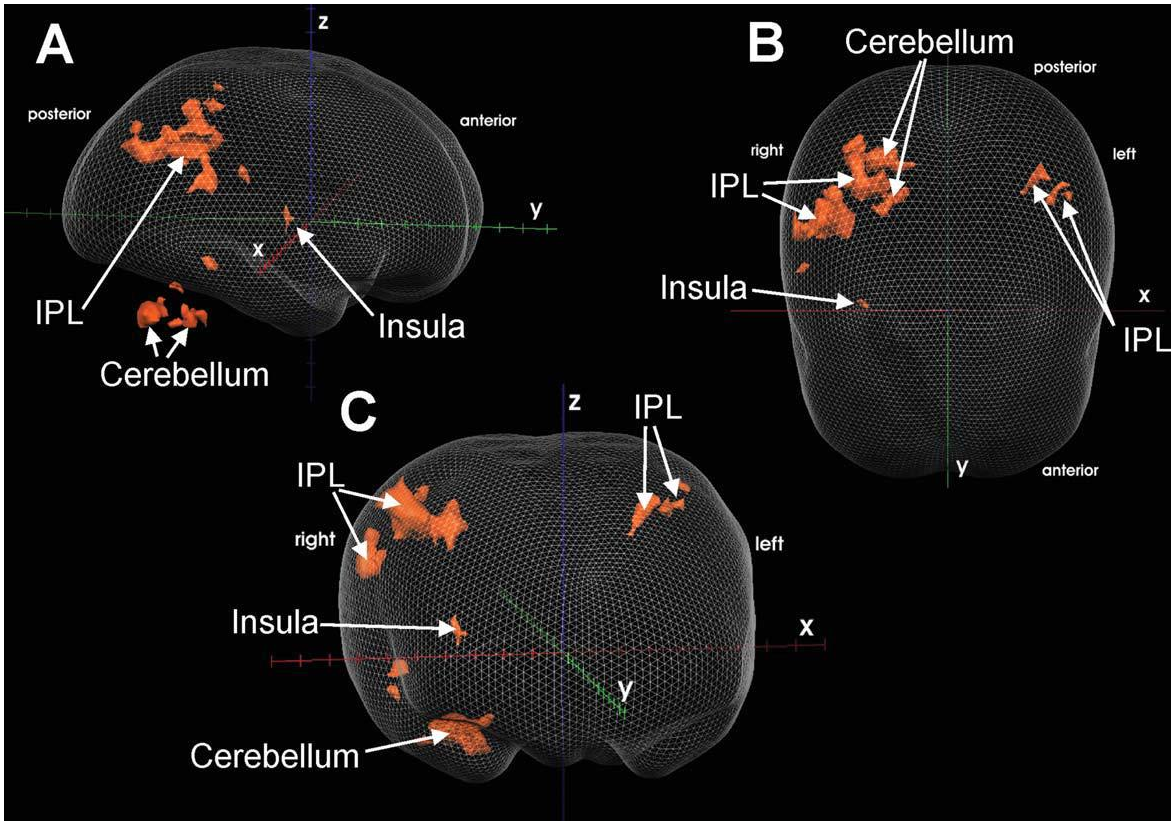

Figure 3 Lateral (A), top (B), and frontal (C) view of a glass brain, showing the supraspinal activations of 12 subjects during the DRAIN 1 condition at random effects level with $t = 4.4$ ($P = 0.001$). Only clusters ≥50 contiguous voxel are shown. For detailed description and coordinates of activated regions, see **Table 2**. Talairach axes (*x*, *y*, *z*) are displayed for easier orientation. IPL = inferior parietal lobe.

DISCUSSION

NERVE FIBER, COLD RECEPTOR, AND CLINICAL CONSIDERATIONS

Cold sensation can be perceived in the urinary bladder as distinct from distention. This is known since bladder cooling experiments have been used in urodynamic investigations [10, 16-18] and specific cold receptors (TRPM8) have been discovered on bladder afferents in the urothelium and suburothelium [11, 19].

Although Geirsson and Fall postulated that the bladder cooling response in humans is mediated by unmyelinated C-fibers [5] and some confirmative evidence of this exists from animal studies (cats;[3]), there is no proof that only C-fibers can transmit cold sensation in the human LUT. Some authors indicate that cold sensation is also transmitted via Aδ-fibers [20]. In addition, TRPM8 receptors have been found on both unmyelinated and myelinated bladder afferent fibers [11].

Therefore, our fMRI findings may not reflect pure C-fiber activation, but rather may predominantly indicate TRPM8 activation. As TRPM8 receptors become active at temperatures below 25°C [11], it might also explain why cold sensation recruits a different cerebral network as compared to bladder filling with warm (body-temperature) saline.

The reason for the existence of cold sensitive receptors in the LUT is still unknown. It has been proposed that they may have the same role in the regulation and maintenance of a stable central core temperature as other thermoreceptors found elsewhere in the body [5, 21]. This is supported by the fact that body cooling is usually associated with increased diuresis, and thus, the bladder cooling reflex has presumably evolved to help relieve the thermal ballast in the bladder during cooling stress [5].

BRAIN NETWORK OF BLADDER THERMAL SENSATION

Although immunohistochemical studies have revealed that cold responsive neurons project to the superficial laminae (I & II) of the dorsal horn in the lumbar and sacral spinal cord of rats [22], little is known about the supraspinal central representation of bladder cold stimulation. A single PET study exists investigating cerebral response to ice water instillation into the bladder of six subjects, which showed activations of a network including the ACG, inferior and middle frontal gyrus, IPL, hippocampus, and crus cerebri [12].

In our paradigm, we used repetitive bladder cooling, taking into account the exact time until bladder cold sensation was perceived by the subjects. This is important, as our results indicate that the cold saline (4–8°C) becomes already warmed to 18°C within 18–24 s after infusion. This is not surprising, considering that the bladder lies deep within the inferior abdomen. For this reason, we performed the test run prior to the functional MR-scanning, to adapt the timing of the scan paradigm accordingly.

The behavior of the BOLD signal during each condition (see **Figure 4**) matched the subjective sensations during the test runs (**Table 1**). During SENSATION, the feeling of bladder cooling started and increased, as did the BOLD signal. During DRAIN 1, where the greatest activation was observed,

subjects indicated a further increase of bladder cold sensation, followed by a slow and constant decline of the cooling sensation until it disappeared toward the end of DRAIN 2, where no activation was observed. The fact that bladder cold sensation continued to increase during DRAIN 1, although the bladder started to empty, might be due to the fact that the bladder drainage lasted slightly longer than the filling and therefore, some amount of cold saline was still in the bladder during DRAIN 1. Another reason might be an after-cooling effect with a delayed spreading of cold sensation in the bladder tissue. This can also be observed on the skin, where cold sensation does not disappear immediately following removal of the cooling source.

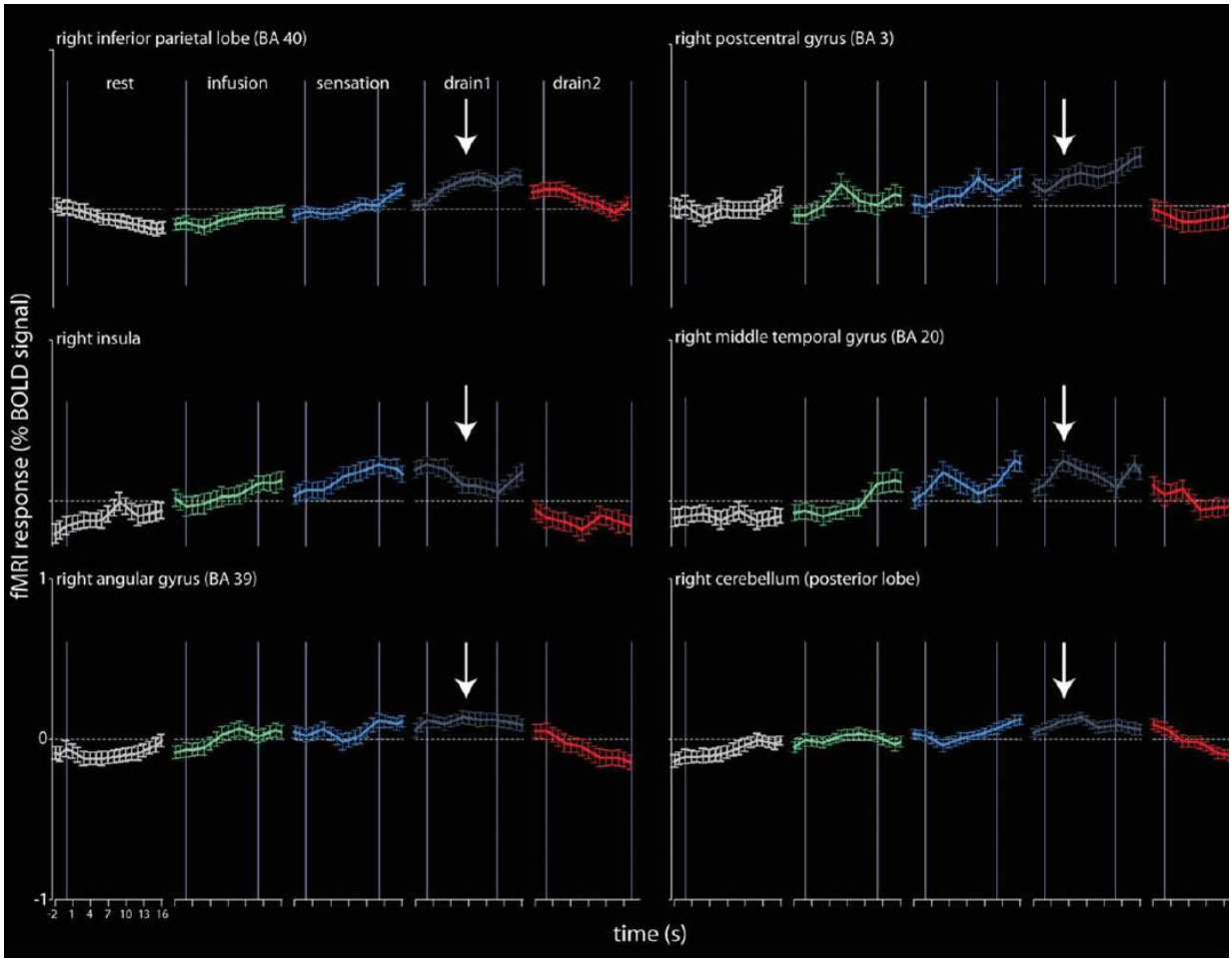

Figure 4 The progression of the BOLD signal during each experimental condition for all activated regions found in the group analysis (**Table 2**). The BOLD signal was extracted from a sphere (10-mm diameter) around the peak activation in each region for the contrast DRAIN 1–REST.

Assessment of subjective sensations has not been performed during the functional MR-scans, which might be a limitation of this study, and slight shifts in the timing of sensations in relation to our scan paradigm cannot be entirely excluded. However, assessment of subjective sensations during ongoing scanning would further complicate the experimental procedure and might be a source of additional artifacts.

After functional MR-scanning, all subjects confirmed a feeling of repeated bladder cooling that matched the timing and sensations felt during the test run.

The SENSATION condition was not prolonged to encompass the entire period of cooling sensation, as this would have caused distention of the bladder. With the 14- Fr Foley catheter and a filling time of 18–24 s used in the present study, only 80–90 ml saline could flow into the bladder. Thus, with a mean bladder volume of SDV 579.7 ± 179.2 ml, at most only a first sensation of filling was perceived by the subjects, avoiding further distension of the bladder [2].

Similar to the PET study of Matsuura et al., the most prominent and earliest response during bladder cooling was found in the IPL (BA 40; [12]). In contrast to this study, reporting unilateral (left) IPL activation, we observed bilateral activation with a right hemispheric dominance. The IPL is implicated in a series of functions including spatial attention, multimodal sensory integration, oculomotor control, and also in various higher cognitive functions like integrative motor planning and interoception [23-27]. Especially the parietal operculum has been implicated in interoception [26]. The plausibility of the specialization of the right IPL in the representation of internally/ self-generated acts finds support in studies reporting a specific involvement of this area in distinguishing self-produced actions from those generated by others, and studies showing that lesions to this area lead to the loss of corporeal awareness [23]. As bladder cooling is a visceral sensation, it is not surprising that especially areas involved in corporeal awareness and self-perception are activated.

Another important area, involved in viscero-sensory function, processing of nociceptive inputs, and interoceptive awareness of visceral sensations, is the insula [28]. In contrast to Matsuura et al., who did not find insula activation during bladder cooling, we observed right-sided insula activation [12]. In addition, thalamic activation was found in 3/4 of the subjects. The insula, thalamus, and secondary somatosensory cortex (BA 3, **Table 2**) are considered to be the key brain structures for somatic thermal perception [29, 30]. Although the bladder is a visceral organ, similarities between visceral and somatic thermal perception have been previously reported [31]. Moreover, cold water flowing through the urethra might have also contributed to activations in these areas. However, this effect seems to be low, as no activation could be detected during INFUSION.

In a recent fMRI study with urgency incontinent subjects, Tadic et al. demonstrated increased activity in the insula, temporal and parietal lobes, in areas very similar to the ones found during thermal C-fiber stimulation in our study (**Table 2**; [32]). This may further suggest the potential role of C-fiber function in the etiology and pathogenesis of OAB and urgency incontinence [1, 2, 6-8, 11].

Unlike Matsuura, we did not observe significant activation in the ACG or frontal regions in our group results [12]. However, the single subject analysis revealed ACG activations in half of the subjects and some frontal activation in each of the subjects. Nevertheless, these activations were too scattered to result in any significant activation in the group analysis. In previous studies, the ACG was implicated in the sensation of bladder fullness (i.e., strong desire to void, urgency) and micturition control including pelvic floor contractions [33-37]. The frontal cortices are thought to be responsible for planning complex social behaviors, and activation in the right inferior frontal gyrus has been implicated in decision making and motivational control, such as whether or not micturition should take place (Kavia et al., 2005). However, our study did not involve any specific task like pelvic floor muscle contraction,

micturition, or withholding or attempted micturition, which might explain the low or scattered activation of ACG and frontal areas.

In our study, we did not detect pontine activation in the group analysis, similar to the findings of Matsuura et al. (2002). However, most subjects showed activations in the pontine region in the single subject analysis. The pontine micturition center (or M-region) and the pontine continence center (or L-region; [28]) show activation during micturition and storage-related tasks, respectively [33, 37, 38]. However, filling the bladder with only small amounts (≤100 ml) of cold water without voiding or withholding of urine, as in our experimental condition, was not expected to elicit pontine activation in these specific areas.

Activation of the PAG area was observed in 9 of the 12 subjects in the single subject analysis, but not in the overall group analysis. The PAG is an important relay station for sensations arising from visceral organs [26, 39] and therefore, a more robust activation of this region was expected. Failure to detect activation in the group analysis may be due to methodological limitations related to inadequate spatial and temporal resolution of both fMRI and PET, and also increased susceptibility to movement-related artifacts in the brainstem region.

In general, activations and deactivations appeared to be predominantly located in the right hemisphere, which indicates that processing LUT sensations may show a hemispheric dominance, as has been previously reported [33, 36]. Hemispheric asymmetry is not uncommon in cerebral control and coordination of human body functions and has been observed for other functions like tactile discrimination [40] and human respiratory control [41].

CONCLUSION

In conclusion, our findings indicate that bladder cooling involves a different supraspinal network than the one reported to be involved during bladder distention in previous PET and fMRI studies. The main difference is a pronounced parietal activity during bladder cooling, with only scarce frontal and brain stem activity. This suggests that bladder cooling is processed in the brain differently than bladder distention. These findings might provide new insights for further research in patients with OAB and painful bladder syndromes, as bladder cold receptor expression and bladder cold perception seem to be altered in those patients. Whether these differences in brain activation are related to the conduction of signals via different afferent fiber types (Aδ- vs. C-fibers) and their supposed specificity toward certain sensations (e.g., distention vs. cold temperature) remains unclear, as cold receptors have been found on both fiber types. More basic research will be necessary to further elucidate the exact type of afferent fibers responsible for transmitting various bladder sensations in humans.

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